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STUDIES TO DETERMINE METHODS FOR CULTURING THREE FRESHWATER ZOOPLANKTON SPECIES

Dan B. Martin, et al

Bureau of Sport Fisheries and Wildlife

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16. ABSTRACT: Studies to determine laboratory methods for culturing unispecific populations of Bosmina longirostris, Chydorus sphaericus and Cyclops bicuspidatus thomasi were carried out. These cultures are to provide a source of animals to be used as live food for fish and as bioassay test organisms. B. longirostris was not successfully cultured. High mortalities, apparently associated with the phenomenon of "air-locking," always occurred during handling in the laboratory. C. sphaericus was successfully maintained in relatively dense cultures (approximately 1,000 per liter) using a mixture of dried foods, less than 37 microns in size. One-fourth of the standing crop was harvested each week without apparently reducing the production in the culture. C. bicuspidatus thomasi could be grown using both dried food and live Paramecium multimicronucleatum as an energy source. However, the latter resulted in higher standing crops. Total standing crop as well as the proportion of each life stage in the population fluctuated greatly in the C. bicuspidatus thomasi cultures. Both C. bicuspidatus thomasi and C. sphaericus were grown at 15° C, at a light:dark cycle of 12:12 hours, and in a synthetic medium of known chemical composition. C. sphaericus was recommended as being best suited for live fish food and as a bioassay test animal.

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May 1975

STUDIES TO DETERMINE METHODS FOR CULTURING THREE FRESHWATER ZOOPLANKTON SPECIES

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ABSTRACT

Studies to determine laboratory methods for culturing unispecific populations of Bosmina longirostris, Chydorus sphaericus and Cyclops bicuspidatus thomasi were carried out. These cultures are to provide a source of animals to be used as live food for fish and as bioassay test organisms. B. longirostris was not successfully cultured. High mortalities, apparently associated with the phenomenon of "air-locking", always occurred during handling in the laboratory. C. sphaericus was successfully maintained in relatively dense cultures (approximately 1,000 per liter) using a mixture of dried foods, less than 37 microns in size. One-fourth of the standing crop was harvested each week without apparently reducing the production in the culture. C. bicuspidatus thomasi could be grown using both dried food and live Paramecium multimicronucleatum as an energy source. However, the latter resulted in higher standing crops. Total standing crop as well as the proportion of each life stage in the population fluctuated greatly in the C. bicuspidatus thomasi cultures. Both C. bicuspidatus thomasi and C. sphaericus were grown at 15° C, at a light:dark cycle of 12:12 hours, and in a synthetic medium of known chemical composition. C. sphaericus was recommended as being best suited for live fish food and as a bioassay test animal.

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SECTION I

INTRODUCTION

Laboratory culture of zooplankton is still in the early stages of development. At present, it seems as if most efforts have proceeded along two divergent lines of investigation. The first is exemplified by the work of Murphy (1970), Provasoli et al. (1970), D'Agostino and Provasoli (1970), or Taub and Dollar (1964), in which the zooplankters are grown in bacteria-free systems, under rigorously defined chemical and physical conditions. Such studies are valuable in determining nutritional requirements of the animals or in determining certain physiological responses to changing environmental variables. However, monoxenic culturing methods are often tedious and do not lend themselves to the production of large numbers of organisms for routine use. Furthermore, the culture conditions are highly artificial with respect to the natural environment. In contrast to these rigidly controlled culture techniques, organisms are often cultured under conditions where many of the important environmental properties are poorly defined. Typically, the medium employed is "pond" or "lake" water. Often, the food consists of mixed algae, bacteria, protozoa or detritus (both living and non-living). These methods are useful for holding organisms for short periods of time for a variety of test purposes. Over long periods of time, populations in such cultures tend to fluctuate in an unpredictable (and often inconvenient) manner. These methods are often difficult to repeat in other laboratories.

The purpose of this study was to develop methods for culturing Cyclops bicuspidatus thomasi, Bosmina longirostris, and Chydorus sphaericus. These three species were chosen because they are known to be important foods for fish at first feeding (Siefert, 1972).
Culture methods are needed to provide:

1. An adequate and natural food supply for larval and juvenile fish being used in chronic toxicology testing programs.
2. A reliable supply of these zooplankters to be used for the determination of water quality criteria (bioassay).

Specifically, the culture methods would have to:

1. Be capable of supplying a constant (and predictable) number of organisms.
2. Produce organisms that were morphologically and physiologically equivalent to individuals found in natural populations.
3. Be well enough defined so that they could be reproduced by any investigator.

The following steps were considered necessary to accomplish these objectives:

1. A method that can be considered "adequate" for maintenance of a limited population must be developed.
2. The method must be simplified and improved, and the important variables defined.
3. The optimum conditions for production must be determined.

Once suitable culture methods became available, they could also be used to study the effects of certain environmental variables on the life history and ecology of the organisms. Such studies were the secondary objective of this project, however, most of the effort was spent on evaluation of mass-culture methods. The degree of progress varied with each of the three species, and additional data are needed in several areas. It is hoped that the information in this report will serve as a starting point for future efforts in culturing zooplankton.

SECTION II

GENERAL METHODS

All experiments, unless otherwise stated, were carried out in Percival Model I35L environmental chambers. Light intensity was the same for all experiments, and consisted of two General Electric, 20 Watt, cool white fluorescent lamps per chamber. Photoperiod was constantly set at a 12:12-hour light:dark cycle. Temperatures within each chamber were monitored continuously with a Weksler, Type 12M3A5A recording thermometer.

The culture medium was varied from time to time, and these specific conditions will be described later. However, frequent reference is made to "filtered Missouri River water". This is water taken from the Missouri River at Gavins Point Dam, diluted with distilled water to a total hardness of about 50 mg/liter, and filtered through a "Millipore" Type HA filter (0.45 micron pore size) before use.

Glass containers with a high surface to volume ratio were used for all experiments. This was to provide for maximum oxygen exchange at the air-water interface and reduce the possibility of oxygen depletion. Except where noted, no aeration was provided in any of the cultures. Dissolved oxygen was not monitored continuously in the cultures but periodic "spot checks" were taken with a Yellow Springs Model 54 oxygen meter.

One method was used consistently throughout the studies for determining the population density of organisms in the cultures. First, the culture was gently stirred to disperse the organisms evenly, and then a 500 ml sample was rapidly withdrawn. All organisms in the 500 ml sample were counted and the result used to estimate the standing crop in the culture.

SECTION III

BOSMINA LONGIROSTRIS

Attempts to isolate Bosmina longirostris from field populations were begun in July 1972. Two sources of specimens were found:

1. A shallow, marshy area near the headwaters of Lewis and Clark Lake, comprising part of the area known locally as the "Springfield Bottoms".
2. Several of the fish rearing ponds at the Gavins Point National Fish Hatchery near Yankton, South Dakota.

Since these two areas are separated by only about 56 kilometers, and since both receive water (and plankton stock) directly from the Missouri River, no attempt was made to separate organisms from the two habitats in any of the experiments. B. longirostris were abundant in the plankton from both localities from mid-June until mid-September.

The first method used to collect and transport field populations of B. longirostris has been used successfully for other cladocerans and copepods. First, the zooplankton are concentrated by slowly towing a Wisconsin plankton net (with removable bucket) for 10 to 20 meters. Without completely draining the water from the bucket at the end of the tow, the bucket is removed and the contents placed in an insulated 20-liter plastic bucket containing water from the field source. In the laboratory, aliquots are siphoned from the bucket and plankton are reconcentrated in the Wisconsin-type plankton cups. This concentrate is then used as the source for stock cultures. Stock cultures are started by removing 10 to 20 active adults with a small pipette, and placing them in 2,000 ml of medium.

After several attempts, it became apparent that B. longirostris cultures could not be obtained in this manner. Virtually all of the specimens collected would be air-locked and floating on the surface by the time the laboratory concentrates were prepared. Repeated attempts to insure that the B. longirostris never came in contact with the air-water interface did not succeed. Observations made during these later attempts indicated that shortly after collection, numerous B. longirostris would be swimming normally in the water. When these organisms were left in any kind of container, they would eventually swim to the surface and air lock. Therefore the majority of the air-locked animals were not a result of the mechanics of the procedure, but resulted primarily from this swimming behavior.

Eventually, one modification of the method did allow organisms to survive long enough to be placed alive in containers in the laboratory. This involved putting about 10 liters of ice into the 20-liter bucket along with the water from the field source. The change from 25-30° C to about

0° C apparently reduced the activity of the organisms sufficiently to prevent them from swimming to the surface and air-locking.

No laboratory populations of B. longirostris could be maintained, either in unispecific or mixed cultures, during July and August. Environmental chambers were not received until late August, and all attempts to keep populations at room temperature (above 24° C) failed. After each attempt, B. longirostris would be found floating on the surface of the water within a few hours. After the environmental chambers arrived, it was noted that a few B. longirostris were surviving in some of the mixed cultures, at lower temperatures. These cultures were obtained by the ice-water method but instead of individuals being isolated into pure cultures, the mixed populations were simply placed, (with a minimum of handling), in 4-liter containers of pond water. Repeated attempts were made to establish unispecific cultures of B. longirostris from these mixed species populations. A partial list of the conditions that were tried follows:

1. Temperature - 10° C; 15° C; 20° C.
2. Water Movement - In several experiments, the water in the cultures was slowly agitated with a laboratory stirrer. In other experiments, circulation was achieved with an air stone. Continuous as well as intermittent water movement was used.
3. Food - a) Enterobacter aerogenes (#15-5030 Carolina Biological Supply Co.). Liquid cultures (100 ml) were grown aseptically in Nutrient Broth (Difco Laboratories, 1953) at room temperature for 7 days. Cells were harvested by centrifuging. Cells were resuspended in distilled water, rinsed, centrifuged again, and stored frozen in distilled water.

   b) Enterobacter cloacae (#15-5032 Carolina Biological Co.). Cultures were grown and harvested in the same manner as that described for E. aerogenes.

   c) Chlamydomonas reinhardtii (#90 Indiana University Culture Collection). This alga was grown in 2,000 ml, bacteria-free, batch cultures using media and methods described by Starr, (1964). Sufficiently dense cultures were obtained so that concentration of the cells was not necessary. Media and cells were added directly to zooplankton cultures.

   d) Chlorella ellipsoidea (#20 Indiana University Culture Collection). This organism was cultured aseptically in 2,000 ml batch cultures using methods described by Starr, (1964) and was added directly to zooplankton cultures.

   e) Cerophyll (Cerophyll Laboratories, Inc.)

   f) Brewer's Yeast (Schiff BioFood Products, Inc.)

   g) TetraMin tropical fish food (Tetra Sales, Inc.)

   Cerophyll, brewer's yeast, and Tetra were ground into powder using a "micro-mill" (Chemical Rubber Co). The powder was then separated into the following size categories using an

Allen-Bradley "Sonic-Sifter": less than 37 microns; 37-53 microns; 53-75 microns; 75-90 microns; and 90-105 microns.

Stock solutions containing each size of each food were prepared by adding known quantities to distilled water and were kept frozen when not in use. Feeding solutions were prepared fresh every week from stock solutions.

h) LIV (Farm and Wildlife Products, Inc.)

i) Trout Starter (Glencoe Mills Co.)

j) Salmon Starter (A preparation acquired from the Gavins Point National Fish Hatchery).

LIV, Trout Starter, and Salmon Starter were ground into powder in the "micro-mill"; but they could not be separated into size categories in the "Sonic-Sifter" because of their tendency to lump together. Instead, the powder was first added to distilled water and the resulting mixture was passed through a series of standard soil testing seives (W. S. Tyler, Inc.). The same particle size ranges as those described earlier were obtained. The quantity of food in each stock solution was determined by filtering a sample onto a glass-fiber filter and drying to a constant weight at 100° C. Feeding solutions were then prepared fresh each week by diluting stock solutions appropriately. The latter were stored frozen.

k) Bacterized - Cerophyll. This was prepared by boiling 1 gram of Cerophyll powder for about 1 hr in 1 liter of distilled water. After the solution had cooled to room temperature, it was inoculated with Enterobacter aerogenes. This was the same food that was used to culture Paramecium multimicronucleatum that in turn was used for food for Cyclops bicuspidatus thomasi. (See later section).

Each of these foods (a-k) were used separately, in various concentrations, and in several combinations.

4. Medium - Hatchery pond water from which the organisms were collected, and Missouri River water. The water was filtered through a Millipore HA membrane (0.45-micron pore size).

Organisms could not be obtained from the field between October 1972 and May 1973, and the experiments just summarized depleted the populations in all but one of the mixed stock cultures. In June 1973 the decision was made to terminate efforts for culturing B. longirostris. The remaining culture containing B. longirostris was maintained until November 1973. Zooplankton populations in this culture had been sampled biweekly (between August 1972 and November 1973). Filtered Missouri River water was added to maintain the culture volume at 2,000 ml.

The history of this culture is shown in Figure 1. Initially, the culture contained: C. bicuspidatus thomasi, 170 per liter; Simocephalus sp., 107 per liter; Diaptomus sp., 2 per liter; Ceriodaphnia pulchella, 72 per liter; Pleuroxus denticulatus, 7 per liter; and B. longirostris, 5 per liter. The original medium was unfiltered pond water (from the source of the organisms), and no food was added during the first 14 weeks. From week-14 to week-30, a mixture of C. reinhardtii and C. ellipsoidea was added once a week. During the remaining 30 weeks, additions of algae were discontinued, and a mixture of LIV, Cerophyll and brewer's yeast (less than 37 microns) was added three times a week.

Only three of the original species persisted throughout the life of the culture. P. denticulatus increased steadily during the first several weeks, reaching a maximum of 1,137/liter at week-22; and fluctuated thereafter between about 200 and 800 per liter. C. bicuspidatus thomasi was at a minimum of 36/liter at week-12 and reached a maximum of 847/liter at week-30. B. longirostris fluctuated between 5 and 75 per liter during the first two feeding phases (i.e., no food and algae); while during the last phase, an increased standing crop (between 42 and 265/liter) was observed.

Diaptomus sp. reached a maximum of 4/liter at week-4 and was not found again. C. pulchella increased initially to a high of 401/liter at week-2, then declined rapidly and was not found again after week-10. Simocephalus sp. also reached a maximum at week-2 (491/liter) and then declined. This last species persisted in the culture for 36 weeks, but was not found after that time.

The significance of Figure 1, as it relates to the original objectives of the study, is negligible. It has been included for three reasons. First, it illustrates the present status of this investigator's ability to culture B. longirostris, after one year of rather intensive effort. Second, after looking at hundreds (maybe thousands) of dead or dying B. longirostris floating on the surface of uncounted cultures, the impulse to include something in this report about living B. longirostris could not be resisted. Third, Figure 1 suggests a principle known as enrichment culturing that may well be worth pursuing in other studies of zooplankton ecology and/or culture. This technique was first used by Winogradsky and Beijerinck in the early 1900's to isolate micro-organisms important in geochemical transformations, and the method has since been applied extensively in bacterial and algal studies. Essentially, it is an application, on a microscale, of the Darwinian principle of natural selection. The investigator devises a culture with a particular, defined set of conditions, inoculates it with a mixed population, and then ascertains which species come to predominate as time continues. Since their predominance is caused precisely by their ability to flourish in the "enrichment medium", the same species can usually be isolated and

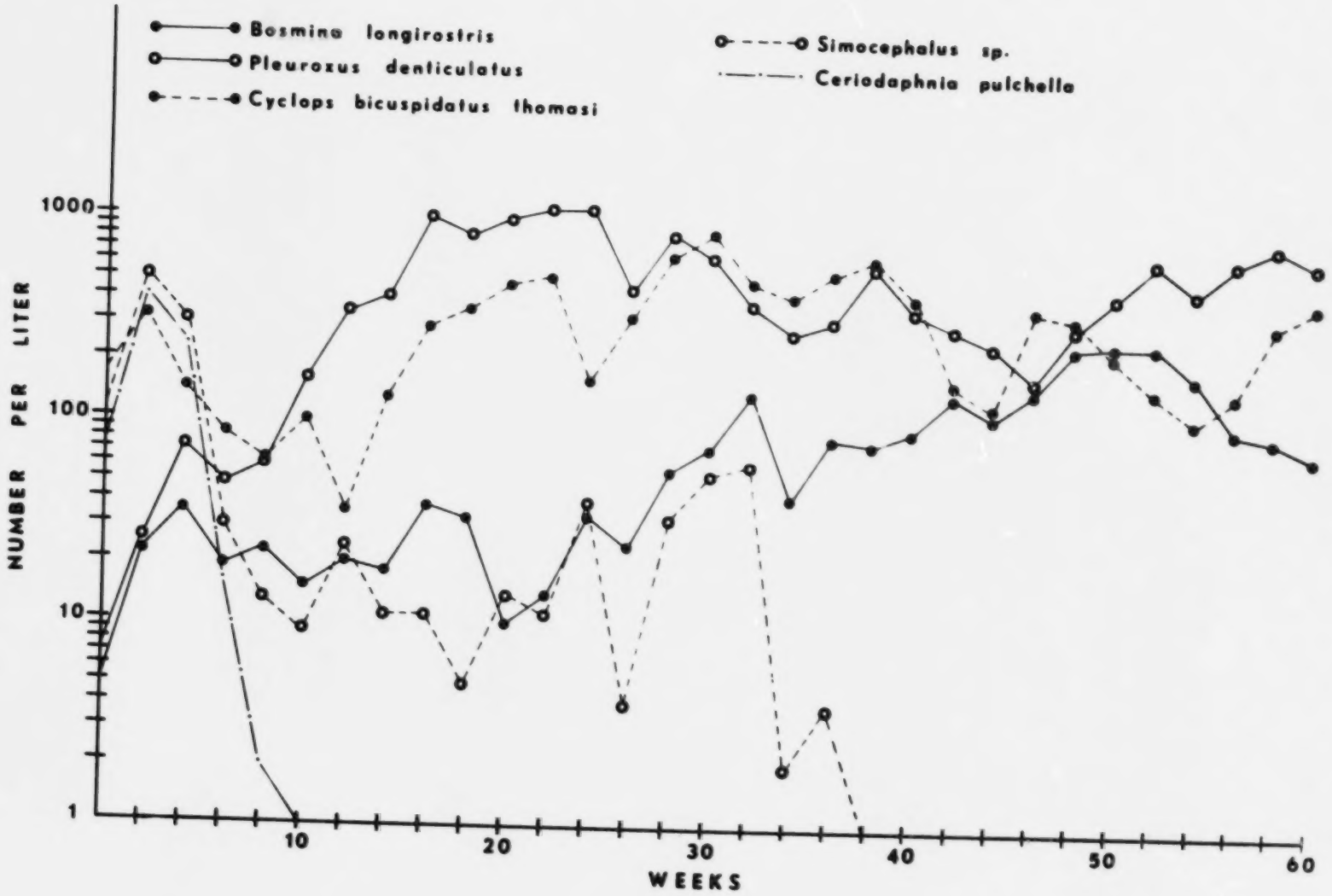

Figure 1. Changes in population density (No./liter) in a mixed zooplankton culture containing Bosmina longirostris.

maintained in a medium of this same composition. An application of this principle will be shown in the next section.

The reasons for the repeated failure of all efforts to establish unispecific cultures of B. longirostris seem to all relate to the phenomenon of air-locking. The carapace of many cladoceran species is hydrophobic, and thus, when the animals are exposed to an air-water interface they become trapped by the surface tension and are unable to submerge themselves again. In field collections of plankton, air-locked Daphnia sp. are often seen floating on the surface. These animals become air-locked during the collection process. It is not really known how frequent air-locking occurs in natural populations under field conditions.

The tendency for B. longirostris to become air-locked during laboratory manipulation has been observed by other investigators attempting to culture this organism. Richard Applegate, South Dakota State University, (personal communication), has cultured a number of zooplankton species during the past several years using pond water and mixed cultures of natural foods. He has repeatedly tried to establish cultures of B. longirostris only to find that all of the organisms would appear air-locked at the surface of the cultures, usually during the first few hours following isolation. He has not been successful in establishing cultures of B. longirostris under any conditions. James S. Murphy, Rockefeller University, has successfully cultured about 14 species of the family Daphnidae, under rigidly defined, monoxenic conditions (Murphy 1970). B. longirostris is not a member of the family Daphnidae, but Bosminids and Daphnids are closely related (i.e., they belong to the tribe Anomopoda). In Murphy's medium large quantities of protein (Bovine albumin, fraction v, at 200 mg/liter) are added to decrease the hydrophobic condition at the surface of the carapace, and hence reduce the tendency for the animals to air-lock. Murphy reported (personal communication) however, that he had repeatedly tried to establish cultures of B. longirostris, using the technique that proved successful with the other cladocerans, and that he was not able to do so. In Murphy's technique, cultures are not started by isolating live individuals. Instead, eggs are removed from the parent's brood pouch, sterilized, and subsequently hatched in a bacteria-free medium to produce the first generation. Murphy's experience suggests that even if B. longirostris can be kept alive through the first few critical hours, problems in maintaining cultures may still exist.

The possibility of unsuitable culture conditions such as light, temperature, container size, medium composition, and food supply cannot be completely disregarded as reasons for failure to establish unispecific cultures. However, the prolonged maintenance of mixed cultures containing B. longirostris (such as the one described in Figure 1), suggest that the physical and chemical environment was within the limits of tolerance

for survival of this organism. Information on food supply is very limited because there was no way of determining exactly what the B. longirostris were utilizing in the mixed cultures, and there was no survival in any of the experiments using defined food. It seems unlikely that either quantity or quality of food was the major reason for mortality. The concentration, size, and nutritional diversity of the food used in these experiments covered a wide range of conditions, and seemingly included those considerations reportedly important for other cladocerans (Murphy, 1970; D'Agostino and Provasoli, 1970; Burns, 1968).

In spite of the difficulties encountered in the present study and the reports received from the aforementioned investigators, there are still a few reports in the literature of apparent success with respect to culturing B. longirostris. Zhdanova (1969) reported several characteristics of growth and development for B. longirostris and B. coregoni in culture. Semenova (1968) studied the effect of temperature on various life stages of B. coregoni, and Burns (1968) included B. longirostris in her study of the relation between food particle size ingestion and body size for several cladocerans. These studies all had one thing in common. The methods of obtaining and manipulating individuals as well as the actual conditions of the culturing were poorly described. All the studies used local natural water which contained a portion of the original particulate matter (living and nonliving). The two Russian investigators used mixed algal cultures (predominately Chlorella vulgaris) as food, and culture vessels with a volume of 50 to 100 ml. Burns used 250 ml experimental cultures and reported that a high percentage of the test animals failed to ingest any of the experimental food. Careful study of these papers failed to reveal any factors not thoroughly explored or considered in the present experiments. However, long-term maintenance of individuals under defined conditions was not the objective of any of these studies, but they do suggest some degree of success at short-term maintenance.

On the basis of our experience it does not appear as if B. longirostris is a suitable organism to use as live fish food or for bioassay test purposes. The requirements for large-scale culturing are unknown but it now appears that these requirements, when known, will be more complex than those for other common zooplankters. Unless the problems of air-locking are solved, there will be large mortalities when individuals or entire populations are manipulated experimentally. This factor greatly reduces the desirability of the species, since high mortalities in the control treatments of a bioassay tend to obscure the effects of the variable being tested. These variable and extreme characteristics of mortality would also complicate any attempts to regulate a constant and/or uniform food supply to fish cultures. In the following section, a method is described for maintaining relatively dense cultures of Chydorus sphaericus, a similar organism.

SECTION IV

CHYDORUS SPHAERICUS

During attempts to establish unispecific cultures of B. longirostris, a procedure for maintaining relatively dense populations of Chydorus sphaericus was discovered. Numerous collections of mixed zooplankton populations were brought into the laboratory during the summer and autumn of 1972. These mixed assemblages were placed in 2-liter containers and subsequently treated in a variety of ways. These were simply trial and error methods of looking for a combination of conditions that would be suitable for B. longirostris culturing and were also an application of "enrichment culture techniques". Initially, from 4 to 12 species would be present in a typical culture. As time passed, most of the species would be eliminated and 1 to 3 species would remain. Usually, at the end, the dominant species would be one cladoceran and one copepod.

The history of one of the cultures just described is shown in Figure 2. This culture was started in August 1972 and was maintained for 66 weeks. Originally, the culture contained: (a) C. bicuspidatus thomasi, 148/liter; (b) Simocephalus sp., 31/liter; (c) C. pulchella, 5/liter; and (d) C. sphaericus, 2/liter. Initially, the medium was unfiltered hatchery pond water. The culture was sampled biweekly. Filtered Missouri River water was added to maintain the volume of 2 liters. No food was added for 14 weeks. A mixture of C. reinhardtii, C. ellipsoidea, and E. aerogenes was added to the culture once a week from week-14 to week-30. At week-30, these food additions were discontinued, and a mixture of LIV, Cerophyll, and brewer's yeast (all less than 37-microns particle size) was added three times a week.

C. pulchella was found only in the initial sample and at week-2. Simocephalus sp. increased during the first 4 weeks, then rapidly declined, and was not found after week-6. C. bicuspidatus thomasi persisted throughout the life of the culture, fluctuating between 15 and 404/liter. C. sphaericus fluctuated between 2 and 49/liter during the first phase of the culture (the period during which no food was added). No apparent trend in population density occurred during the second phase (when algae and bacteria were added as food); as numbers during this period ranged between 2 and 24/liter. Following the change to LIV, Cerophyll, and brewer's yeast, the population density of C. sphaericus began to increase. The rise continued until shortly before the culture was terminated. The maximum population density reached was 1,098/liter. Similar results were noted in several other mixed cultures containing C. sphaericus, and it became evident that perhaps a method had been found for maintenance of relatively dense populations of this organism.

Four unispecific, stock cultures of C. sphaericus were started in October 1973. Ten to twenty mature individuals were placed in 4-liter vessels

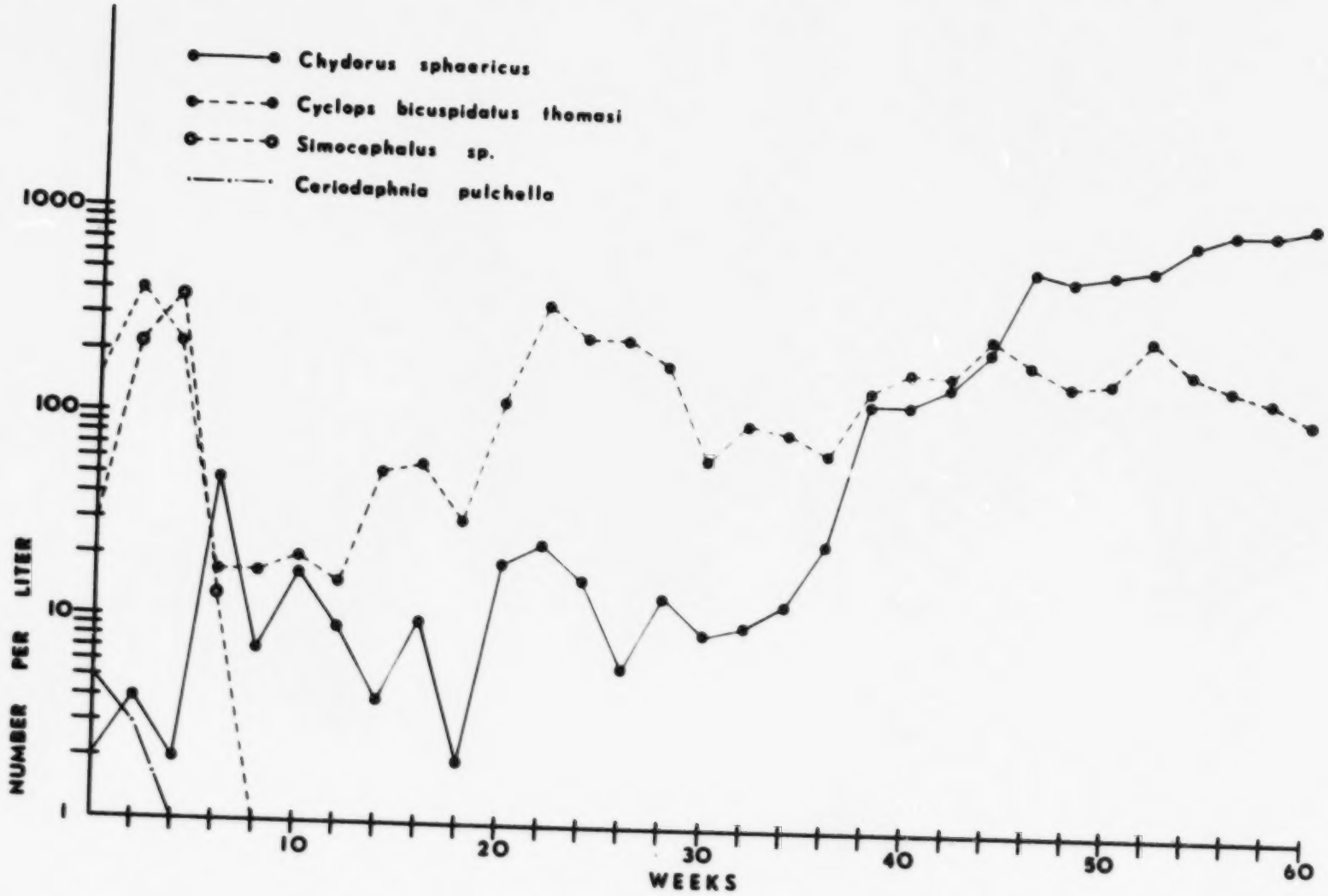

Figure 2. Changes in population density (No./liter) in a mixed zooplankton culture containing Chydorus sphaericus.

containing 2 liters of filtered Missouri River water at 15° C. One milligram each of LIV, Cerophyll, and brewer's yeast (all less than 37 microns particle size) was added to each culture three times a week. The cultures were sampled once per week and fresh medium was added to the container to maintain the 2 liter volume.

A summary of C. sphaericus produced in the stock cultures is found in Table 1. Culture No. 1 was sacrificed to supply stock for another experiment (to be discussed later). Culture No. 2 was discontinued in order to supply stock for cultures at the National Water Quality Laboratory (EPA), Duluth, Minnesota. The remaining two cultures were maintained for 225 days. The average standing crop in the stock cultures ranged from 1,624 to 2,268/liter. One-fourth of the standing crop was removed each week, and it appeared that production was fairly well balanced with the number harvested, since there were not large fluctuations in standing crop after the populations became established. It can be concluded that 200-300 individuals per liter per day can be produced in large cultures under the conditions described for these stock cultures. Time did not allow for additional experiments to determine how closely the present values approach maximum production. Different rates of feeding and harvesting, as well as different temperatures must be investigated to determine conditions for maximum yield.

Table 1. Summary of stock cultures of Chydorus sphaericus.

Culture number	Duration (Days)	Total number harvested	Average harvested (Number per liter per day)	Average standing crop (Number per liter)
1	49	6,713	274	1,918
2	78	9,048	232	1,624
3	225	36,354	324	2,268
4	225	33,685	300	2,100

As soon as it became apparent that C. sphaericus could be grown in a medium of Missouri River water, another experiment was started to determine if a synthetic medium, of defined chemical composition, could be used in place of the natural water. Synthetic water would have wide applicability and may reduce variability in future experiments. The filtered Missouri River water was compared with the synthetic medium described by Sheer and Armitage, 1973. Treatments were set up in the following manner: (a) Treatments 1A and 1B used filtered Missouri River water, while 2A and 2B used synthetic medium; (b) 1A and 2A both received food in the same

manner as that described for the stock culture (i.e., 9 mg/week); and (c) 1B and 2B received one-half the amount of the same foods. One hundred mature individuals were placed in each 2-liter culture at 15° C. Cultures were sampled every week and fresh medium was added to maintain a 2-liter volume. The results of this experiment are shown in Table 2.

The synthetic medium appeared to be at least as good as Missouri River water for culturing C. sphaericus. Population densities at both food levels in the defined medium were higher than those at either of the food levels in the Missouri River water. This situation may have resulted from an uncontrolled variable that appeared near the end of the experiment. Apparently, the synthetic medium was more favorable for the growth of algae. The contribution of the algae to the food supply of the C. sphaericus is unknown, but it could account for the increased standing crops observed in the synthetic medium. Despite the algae problem, it was concluded that the artificial medium was suitable for C. sphaericus and that it should be used in place of natural waters for future experiments.

The quantity of food added also had a measurable effect on standing crops within each medium treatment (Table 2). The lower level of feeding appeared less than that needed for maximum production. It still cannot be determined if the upper level was sub-optimal or excess for this temperature.

Table 2. A comparison of Missouri River water (MRW) and synthetic medium (SM) for culturing Chydorus sphaericus at two levels of feeding.

Culture number	Culture medium	Food (mg/week)	Duration (Days)	Total number harvested	Average harvested (Number per liter per day)	Average standing crop (Number per liter)
1A	MRW	9.0	63	7,030	224	1,568
1B	MRW	4.5	63	3,696	118	826
2A	SM	9.0	70	13,250	378	2,646
2B	SM	4.5	70	8,334	238	1,666

The metabolism of poikilothermic animals is assumed to be temperature dependent. Normally production increases with temperature up to an optimum, above which increased temperature results in decreased production and eventually death. The effects of 10° and 20° C on reproduction and population growth were carried out with C. sphaericus. The experiments were started by placing 25 individuals in duplicate 2-liter cultures. Synthetic medium was used, and feeding was carried out as previously described for the stock cultures. Cultures were sampled each week, and all eggs within the brood pouches of adults were also counted.

Temperature had a marked effect on standing crop and population fecundity (Table 3). There was at least one order of magnitude difference in both the average number of individuals per liter and the average number harvested at the two temperatures. The number of eggs produced by the populations followed the same trends. Temperature did not have any apparent affect on the population age structure. The percent of the total population with eggs was relatively constant at both temperatures, indicating about the same proportion of juveniles and adults in all cultures. The lower temperature did not seemingly impair the ability of the individual animal to produce eggs. Both the average clutch size and the ratio of eggs per animal appeared independent of temperature.

Table 3. The effects of two temperatures (10° and 20° C) on population density and reproduction of Chydorus sphaericus. All cultures 87 days duration.

	20° C		10° C	
	Replicate		Replicate	
	A	B	A	B
Total number harvested	3,784	2,530	234	262
Average harvested (Number per liter per day)	86	58	6	6
Average standing crop (Number per liter)	602	406	42	42
Percent of total population with eggs	19.7	21.6	23.1	18.3
Average clutch size	2.1	2.1	1.6	2.2
Average standing crop eggs (Number per liter)	250	187	14	17
Average eggs per animal	0.4	0.5	0.4	0.4
Total number eggs harvested	1,552	1,162	88	104

These results indicate that the lower temperature reduced the population density by decreasing growth rates and increasing the time for development of each stage in the life cycle. No evidence was found that the lower temperature was detrimental to the individual animal. Keen (1973) reported an egg duration time of about 60 hr at 20° C, and about 300 hr at 10° C for Chydorus sp.

The average standing crop and the number of animals produced per day were less for the 20° C treatments of this experiment than those observed in the previous experiments at 15° C under similar feeding conditions. It is possible that 15° C is more near optimum, and 20° C is too warm for maximum production. It is also possible that since the individual or-

ganisms expend more energy at 20° C, the 9 mg/wk of food was not sufficient in these cultures. The uncertainty in interpretation of these results points up the necessity of further experiments involving the interaction of food supply and temperature. When these relationships are known optimum conditions for mass culturing this organism will be more nearly defined.

In spite of the obvious need for more work, a simple, reproducible, and relatively efficient method for producing large numbers of C. sphaericus has been found. The small size of the organisms make them appear desirable as larval fish food. Furthermore, repeated handling in the laboratory did not result in any significant mortality of individuals. In contrast to our experience with B. longirostris, air-locked C. sphaericus were rarely observed. The low mortalities observed in laboratory cultures under controlled conditions make this species appear particularly desirable for bioassay test purposes.

SECTION V

CYCLOPS BICUSPIDATUS THOMASI

Two 10-liter stock cultures of Cyclops bicuspidatus thomasi were begun in November 1972 by isolating mature males and females with eggs from collections obtained from fish ponds at the Gavins Point National Fish Hatchery. The cultures were maintained until March 1973, using filtered Missouri River water. In March 1973 all of the C. bicuspidatus thomasi were removed from one of the cultures and placed in a similar 10-liter culture containing filtered Lake Superior water. All other conditions, including food supply remained the same in both cultures. These two different stock cultures were maintained from March 1973 until November 1973. In November 1973 all C. bicuspidatus thomasi were removed from both cultures and placed two 10-liter cultures containing the synthetic medium described by Sheer and Armitage (1973) and were maintained until termination of the study in May 1974. Temperature was 15° C throughout the study.

Previous work had shown that mixed protozoan populations, grown in water enriched with sheep manure, could be used as food for culturing C. bicuspidatus thomasi. Therefore, pure cultures of Paramecium sp. were added at a rate of 500/liter three times a week to the stock cultures. This resulted in a total of 15,000 Paramecium sp. per culture per week.

Paramecium sp. were grown in 1- or 2-liter batch cultures, using Cerophyll (1 gram/liter) that had been inoculated with E. aerogenes. Cultures were grown in subdued light at 25° C. Initially, effort was spent in trying to perfect methods of growing Paramecium sp. in continuous cultures (chemostat-type). Several modifications of the techniques described by Gold (1972) were tried, but sufficient numbers could not be obtained. Eventually, batch culturing techniques were relied upon entirely. The quantity of Paramecium sp. used as food was determined by direct count of a 1-ml sample in all experiments, as well as the stock cultures. A 1-ml sample was removed from a well mixed batch culture and the total number of Paramecium sp. in the sample was counted. This number was then used to calculate the volume of culture needed to obtain the desired number of Paramecium sp. for feeding. A significant portion of the bacterized Cerophyll always accompanied the Paramecium sp. into the C. bicuspidatus thomasi cultures at each feeding. This was considered desirable as this bacterial population was probably utilized by the nauplii.

The 10-liter stock cultures were sampled biweekly. The average standing crops of each life stage in the two stock cultures are shown in Table 4. T-tests showed no significant difference between the two cultures with respect to either the standing crops or the percentage composition of the three-life stages, and no significant differences due to changes in medium composition during the life of the cultures. The two cultures appeared as part of the same population throughout their existence. The

standing crop of adults ranged from 2 to 222/liter, and the percentage of adults in the total population varied between 0.7 and 52.1. Copepodites ranged from 4 to 510/liter and comprised 1.8 to 79.2% of the total population. Nauplii standing crop varied from undetectable numbers (on two occasions) to 1,678/liter, and they comprised from 0.0 to 92.6% of the total C. bicuspidatus thomasi present on various sampling dates. The total combined life stages varied from 128 to 1,812 per liter.

Table 4. Average standing crop and average percentage composition for each life stage of Cyclops bicuspidatus thomasi in 10-liter stock cultures. Cultures were maintained at 15° C and were fed 1,500 Paramecium sp. per liter per week.

	Culture No. 1				Culture No.2			
	Number per liter		Percent of total		Number per liter		Percent of total	
Life stage	Ave.	Std.Dev.	Ave.	Std.Dev.	Ave.	Std.Dev.	Ave.	Std.Dev.
Adults	46	36	13.9	12.6	73	67	18.6	14.8
Copepodites	125	105	34.2	24.7	130	142	29.6	23.8
Nauplii	325	442	51.9	32.9	166	86	51.8	31.5
Total	496	435	100.0	-	369	134	100.0	-

Large variations in standing crop as well as shifts in the population age structure occurred in these cultures. At several points the population was dominated by nauplii, while at other times, adults and copepodites were the most abundant forms. These changes in the population may result from the fact that adult and late-stage copepodites are heavily predaceous on the nauplii. This predation was repeatedly observed in our laboratory, and apparently is a common occurrence in natural populations (McQueen, 1969). The familiar cycle of numbers often reported for predator-prey populations involving two species may be operative in these cultures where both predator and prey are of the same species.

Two other characteristics of the stock culture populations did appear to be comparable to those observed in nature. The average clutch size of ovigerous females in the cultures was 29 (range 15-42), and the average ratio of adult females/males was 1.7. Martin and Novotny (unpublished manuscript) found the average clutch size for females in Lewis and Clark Lake was 26 (range 1-78), and the average adult female/male ratio was 1.2.

The efforts to determine optimum culture conditions for C. bicuspidatus thomasi began with an experiment to measure the effects of different levels of food supply on standing crop at 15° C. Three 2-liter cultures were started in filtered Missouri River water by adding 100 adults to each. Each culture was then given a different number of Paramecium sp. three times a week. The three rates of feeding used were (a) 1,000; (b) 1,500;

and (c) 2,000 Paramecium sp. per liter per week. The cultures were sampled weekly. Fresh Missouri River water was added each week to maintain the volume at 2 liters.

At the end of 25 weeks no difference in the standing crops of C. bicuspidatus thomasi could be shown among any of the three food treatments (Table 5). Standing crops and composition for each life stage were comparable to those found in the 10-liter stock cultures, and the large variations within each 2-liter culture were also comparable to the larger cultures. No conclusions could be drawn as to the precise quantity of food necessary to produce maximum population densities. However, it was apparent that 1,000 to 1,500 Paramecium sp. per liter per week was sufficient under these culture conditions to maintain maximum populations, and it is not likely that greater quantities of food would result in greater standing crops.

Table 5. Average standing crop and average percentage composition for each life stage of Cyclops bicuspidatus thomasi in 2-liter cultures fed three different quantities of Paramecium sp. for 176 days at 15° C.

	Culture A		Culture B		Culture C	
Life stage	Number per liter	Percent of total	Number per liter	Percent of total	Number per liter	Percent of total
Adults	112	34.2	84	22.7	90	26.7
Copepodites	160	48.8	168	45.4	150	44.5
Nauplii	56	17.1	118	31.9	97	28.8
Total	328	100.1	370	100.0	337	100.0

Culture A - 1,000 Paramecium sp. per liter per week
Culture B - 1,500 Paramecium sp. per liter per week
Culture C - 2,000 Paramecium sp. per liter per week

Another approach was also used in an effort to determine the amount of food necessary for maximum production. Individual adults were placed in a series of 50-ml containers with about 30 ml of filtered Missouri River water. Different numbers of Paramecium sp. (from 10 to 100) were then placed with each adults. It was assumed that the number of Paramecium sp. needed for mass culture could be determined by knowing the number of Paramecium sp. consumed by an individual, on a daily basis. The same food levels were set up at 10° and 20° C as it was further assumed that food consumption would be greater at increased temperatures. Observations were made every 4 hr on each culture, and the number of Paramecium sp. remaining were counted.

After two trials with this experiment, it was concluded that the adult C. bicuspidatus thomasi would consume all the Paramecium sp. they encountered within the first 24 hr; and this behavior was not related to the temperature used nor to the original number of prey introduced. Apparently, these C. bicuspidatus thomasi were feeding in excess of their requirements for normal maintenance, and this made it impossible to determine the quantity of food required.

Another experiment was set up to determine the effects of temperature on standing crops of C. bicuspidatus thomasi in culture. Three 2-liter cultures containing filtered Missouri River water, and fed 1,500 Paramecium sp. per liter per week, were kept at 10°, 15°, and 20° C. Each culture was started with 100 adults and sampled weekly. Fresh medium was added each week to maintain a volume of 2 liters.

No significant differences (t-test) in the average standing crops of any life stage were found between the cultures at 15° and 20° C (Table 6). A significantly lower average number of adults and copepodites was found in the culture at 10° C ($P > .05$). However, there was no difference in the standing crop of nauplii among any of the cultures. These results show that there was a measurable shift in the population age structure at 10° C, with a higher proportion of juvenile organisms. This would be expected, since development of the various instars would be slower at this temperature (see later discussion on life history). Also, fewer adults would result in less predation on the nauplii, and the ratio of Paramecium sp. to adults would also be greatly increased. It appears that lower temperatures are more favorable for the production of nauplii, and higher temperatures are probably best for overall production. Also, large variations in standing crop and percentage composition of the various life stages discussed for previous experiments were observed in the 15° and 20° C treatments, but there seemed to be less variation in the standing crops at 10° C.

Table 6. Average standing crop and average percentage composition for each life stage of Cyclops bicuspidatus thomasi in 2-liter cultures at three experimental temperatures. Feeding rate was 1,500 Paramecium sp. per liter per week and duration was 210 days.

	10° C		15° C		20° C	
Life stage	Number per liter	Percent of total	Number per liter	Percent of total	Number per liter	Percent of total
Adults	12	3.1	46	8.7	39	7.3
Copepodites	65	16.6	149	28.2	177	33.1
Nauplii	314	80.3	334	63.1	319	59.6
Total	391	100.0	529	100.0	535	100.0

When it became evident that C. bicuspidatus thomasi could be cultured using live Paramecium sp. as food, attention was given to finding a suitable, non-living food. Although, Paramecium sp. satisfied the original criteria for culture methods, there are certain disadvantages to the use of live food. First, it is difficult to determine precisely the quantity of food added at each feeding. The number of Paramecium sp. to be added could be estimated by the method previously described, but since the population density of the protozoans varied considerably in the batch cultures, different amounts of the bacterized Cerophyll would accompany the Paramecium sp. throughout the experiment. For example, if the population density of Paramecium sp. was 80/ml, it was necessary to add about 40 ml of the food culture to a 2-liter C. bicuspidatus thomasi culture to obtain 1,500 Paramecium sp./liter, but if the density of Paramecium sp. was 300/ml only 10 ml of the food culture was necessary. Since it was assumed that the bacterized Cerophyll was important as food for the nauplii, these variations could have had an effect on C. bicuspidatus thomasi populations over time. Unless there is complete control over the quantity of food added throughout an experiment it is difficult to isolate and evaluate the effects of any other experimental variable. The use of live food also has disadvantages in bioassay-type experiments. If the toxicant being tested is detrimental to the food source, this interaction can obscure the more direct effects of the toxicant on the test organism.

An experiment was set up to determine the suitability of certain dried foods for mass culture of C. bicuspidatus thomasi. Three 2-liter cultures were started with 100 individuals in each, in filtered Missouri River water at 15° C. A different combination of dried food was added to each culture (Table 7) at the rate of 9 mg/week. The particle size range of each food was 37 to 90 microns. (A description of these foods and the methods of preparation can be found in the B. longirostris section). The cultures were sampled once a week. Fresh medium was added to maintain a volume of 2 liters.

Table 7. Average standing crop and average percentage composition for each life stage of Cyclops bicuspidatus thomasi in 2-liter cultures fed different combinations of dried food (particle size 37-90 microns) for 254 days at 15° C.

Life stage	Ration No. 1		Ration No. 2		Ration No. 3	
	Number per liter	Percent of total	Number per liter	Percent of total	Number per liter	Percent of total
Adults	36	25.9	10	11.4	15	21.7
Copepodites	47	33.8	53	60.2	21	30.4
Nauplii	56	40.3	25	28.4	33	47.8
Total	139	100.0	88	100.0	69	99.9

Ration No. 1 - LIV, Brewer's Yeast, Cerophyll
Ration No. 2 - Tetra, trout starter, salmon starter
Ration No. 3 - Brewer's Yeast, Cerophyll, Tetra, trout starter

All of the rations were added at the rate of 9 mg per week

All three combinations of dried food supported a population of C. bicuspidatus thomasi for over 36 weeks (Table 7). The proportion of each life stage in the populations was about the same as that observed in the Paramecium sp.-fed cultures, but the total standing crops were lower. Direct observations on individual animals in small containers indicated that adults and late stage copepodites readily devoured particles of dried food. When the particles were placed in the small containers they would sink to the bottom. Eventually, the animals would search the bottom of the container and find the food. It was repeatedly noted that these individuals seemed highly selective in regard to the size of particle they would attack. Small particles were seldom taken. C. bicuspidatus thomasi would select particles in a size range from about the maximum size that they could ingest upwards to large particles. It was not unusual to watch an individual spend several minutes struggling with a particle that was several times larger than it could possibly ingest.

Another experiment was set up to demonstrate the effects of different size food particles on standing crops of C. bicuspidatus thomasi cultures (Table 8). Three 2-liter cultures were started with 100 adults each, in filtered Missouri River water at 15° C. A combination of LIV, brewer's yeast, and Cerophyll (equal proportions of each) was added to the cultures at the rate of 9 mg/week. Cultures were sampled once a week.

Table 8. Average standing crop and average percentage composition for each life stage of Cyclops bicuspidatus thomasi in 2-liter cultures fed different size particles of LIV, brewer's yeast, and Cerophyll. Culture temperature was 15° C, duration was 191 days, and 9 mg of food were added per week.

	Less than 37 microns		37-90 microns		90-150 microns	
Life stage	Number per liter	Percent of total	Number per liter	Percent of total	Number per liter	Percent of total
Adults	2	10.5	20	10.5	28	16.1
Copepodites	2	10.5	78	41.1	34	19.5
Nauplii	15	79.0	92	48.4	112	64.4
Total	19	100.0	190	100.0	174	100.0

A t-test showed significantly lower ($P > .05$) standing crops for all three life stages in the culture receiving food less than 37 microns in size (Table 8). No significant difference was found between the standing crops in the other two treatments. Therefore, food particle size was shown to be an important factor in culture maintenance.

Although, C. bicuspidatus thomasi can be cultured with non-living foods, standing crops in the cultures receiving dried foods have not reached the levels obtained in the Paramecium sp.-fed cultures. Several factors could be responsible. First, it appears that the animals must obtain the non-living foods from the bottom of the cultures, since the larger particles they prefer sink rapidly. This restricts the availability of food since animals usually search for food in the open water portions and along the side walls of the cultures. Secondly, the larger particles break down rapidly. This problem cannot be solved simply by adding more particles since too much organic matter will soon "foul" the cultures. These problems, associated with the stability of prepared foods, have recently been investigated by Balazs, Ross and Brooks (1973), who experienced similar difficulties in mass culturing shrimp. Recently, these investigators have made some progress in the preparation of water-stable foodstuffs.

Another factor resulting in lower standing crops might be an increase in predation by adults on nauplii in cultures without live food. This is probable since, in the absence of Paramecium sp., nauplii would be left as the only moving "natural" food source in the cultures. The proportion of encounters between adults and nauplii would be increased in the cultures without Paramecium sp. There is also a possibility that artificial

foods do not adequately satisfy the nutritional requirements of all life stages of C. bicuspidatus thomasi. Such deficiencies, would result in slower development, decreased reproduction or incomplete development at one or more stages in the life history. All of the tentative presumptions regarding the use of dried food in culturing C. bicuspidatus thomasi represent areas where further research is needed.

In every phase of the work with C. bicuspidatus thomasi one problem that constantly reoccurred was the predation by adults (and late-stage copepodites) on nauplii. This was a variable that could not be controlled or measured in any of the mass culture experiments. Considerable time was spent in trying to devise a method for separating the adults from the nauplii. A chamber was designed to keep the brood stock (adults) in one section and the nauplii in another. Segregation was achieved by size selection. The chamber consisted of an upright 3 liter cylinder with a 175 micron mesh screen on the bottom. Below the screen was attached a 500 ml cylinder and an outlet that was closed off by a piece of rubber tubing and a pinch clamp. The adults were placed in the upper section. A culture volume of about 2 liters was maintained and about 2,000 Paramecium sp. per liter per week were added to the upper section for food. The culture was drawn down at various intervals (ranging from daily to weekly) from the outlet at the bottom. In this manner, the brood stock were retained in the upper section by the screen, but the nauplii were drawn through the screen. Various volumes were drawn out ranging from 500 to 1,500 ml.

These experiments were carried out over about a 6-month period, and at best, the results were erratic. A few nauplii were harvested but the number obtained was not comparable to the number produced. The amount of Paramecium sp. added seemed to be in excess to the amount required by the C. bicuspidatus thomasi since numerous Paramecium sp. would always appear in the harvested portion of the culture. Nauplii were being produced in the upper chambers, because frequent examination of the brood stock showed a good population of adults (approximately 75-150/ liter) including ovigerous females. Probably the nauplii were consumed shortly after hatching and most did not survive long enough to be drawn through the screen and away from the adults. A continuous movement of water through the screen may result in better survival of nauplii but this technique was not achieved.

In addition to the mass culture experiments, the life history of C. bicuspidatus thomasi was studied by observing individual specimens in cultures of 10-100 ml volume. The medium in all of the small culture experiments was filtered Missouri River water. Copepodites and adults were fed Paramecium sp., and nauplii were given small portions of the bacterized Cerophyll (from the Paramecium sp. cultures). In all experiments food was considered to be in excess.

C. bicuspidatus thomasi are known to be bisexual animals with no reports of parthenogenesis. Copulation was observed several times in these studies. In this process, the male grasped the female in the region of the 4th or 5th metasomal segment with his antenule claspers. The joined pair remained attached for 2 to 15 minutes. In our experiments, an unfertilized female was placed in a small chamber with one mature male. After copulation, the male was removed. On most occasions this resulted in viable eggs within 1-3 days following copulation (temperature 15° C). After the first clutch hatched, viable eggs continued to be produced until 4 to 7 clutches hatched. If no further contact with a male was permitted the female would continue to produce unviable eggs. These eggs remained with the female for varying lengths of time, but eventually deteriorated and were dropped. As many as six unviable clutches were produced but the number of eggs usually decreased each time. Ewers (1936) found 3 to 4 consecutive clutches of viable eggs were formed after contact with a male and females would continue to produce ova in the absence of sperm in the seminal receptacle.

Development time of each life stage was temperature dependent (Table 9). The duration of the egg incubation varied from an average of 8.3 days at 5° C to 2.1 days at 20° C. Development through the naupliar stages averaged from 37.3 days at 5° C to 8.0 days at 25° C, and for copepodite stages, 70.4 and 19.4 days at 5° C and 25° C, respectively. Development of an egg to an adult averaged 29.6 days at 25° C, and 116.0 days at 5° C. These developmental periods are similar to those reported by Ewers (1930) who found that 28 to 35 days were required for completion of the life cycle of C. bicuspidatus thomasi at 20° C, and Andrews (1953) who reported an average of 30 days for egg to adult in field populations at about 15° C.

Table 9. Development time (days) for each major life stage of Cyclops bicuspidatus thomasi at five experimental temperatures. (A) Average, (B) Standard deviation, (C) Number of observations.

Life stage		5° C	10° C	15° C	20° C	25° C
Egg	(A)	8.3	5.9	3.6	2.1	2.2
	(B)	1.2	0.9	0.6	0.3	0.7
	(C)	13	11	10	11	26
Naupliar	(A)	37.3	29.7	18.8	10.1	8.0
	(B)	6.4	5.5	5.8	0.7	2.0
	(C)	8	9	12	15	5
Copepodite	(A)	70.4	58.5	43.1	26.6	19.4
	(B)	14.1	16.9	12.2	4.2	3.2
	(C)	2	3	9	6	9
Total	(A)	116.0	94.1	65.5	38.8	29.6

Considerable difficulty was encountered in rearing the individuals in the small containers, especially at the colder temperatures. The eggs would hatch readily regardless of temperature and there did not seem to be any significant reduction in the percentage of eggs hatched at colder temperatures. However, there was a noticeable increase in nauplii mortality associated with the lower temperatures. Food seemed to be plentiful but there was no way of determining if the nauplii were feeding. Eventually, the individuals would cease to become active and die. The reasons for this lack of development could be several. Provasoli and his group have shown that nutritional deficiencies often manifest themselves in the failure of an organism to develop beyond a certain life stage. These workers have also shown that in the case of certain deficiencies (e.g., vitamins) normal development may occur for several generations in laboratory populations, then finally the trace amounts required are finally exhausted and development stops. Ewers (1936) listed the following factors as important in laboratory culture of Cyclops sp.: (1) size of living space; (2) temperature; (3) food; (4) bacteria; and (5) O_2 and CO_2 content. Coker (1934) listed temperature, food, and genetics as the three most important factors on rate of development in Cyclops sp. More recently, Lewis, et al. (1971) and Whitehouse and Lewis (1973) have shown the importance of temperature, food, and container size on culturing Cyclops abyssorum.

Perhaps the most conspicuous property of the C. bicuspidatus thomasi cultures in this study, both large and small, was their striking variability. Part of this variation was due to lack of control over important environmental factors during the culture experiments. However, one should not overlook the wide range of adaptability demonstrated by this species in nature, and the probable genetic basis for some of this observed variation. C. bicuspidatus thomasi is extremely widespread geographically, and its habitat is known to range from the open water of large lakes to the littoral areas of small ponds. In many areas, it is known as a perennial species, and in some localities all life stages are present the year around. Field studies of this species show that it is capable of existence under a wide variety of environmental conditions. These broad limits of tolerance and adaptability are undoubtedly brought into play under culture conditions. Such inherent properties within the species would make it more difficult to demonstrate the effects of some environmental variable (e.g., temperature), and to ultimately determine "optimum" culture conditions. This same variation also seriously weakens attempts to describe the effects of environmental variables from observations on a few individuals (e.g., the small culture experiments).

SECTION VI

GENERAL DISCUSSION AND RECOMMENDATION

An outline of the proposed methods for culturing C. sphaericus, and C. bicuspidatus thomasi will be given in this section along with some other general observations relative to the use of these methods in future studies. The methods outlined are not intended to be interpreted as "optimum" culture conditions. Rather, these methods represent a degree of success in attaining the most efficient means of culturing these species.

We recommend C. sphaericus as a live food for larval fish and as a test organism in bioassay experiments. Large populations of this species can be maintained on a relatively simple diet, under easily controlled environmental conditions. Once established the cultures produce a fairly stable standing crop. Test populations can be handled without significant mortalities. In contrast to C. bicuspidatus thomasi, C. sphaericus seems to have a rather limited range of tolerance to certain environmental factors (e.g., temperature). This makes the latter species a more sensitive experimental animal.

To culture C. sphaericus, one should use glass containers with a volume of at least 2 liters. The effects of container size were not tested in our experiments but 2 liters is sufficiently large. A temperature of 15° C, a light:dark cycle of 12:12 hours, and the medium described by Sheer and Armitage (1973) should be used. Food consisting of equal proportions of LIV, brewer's yeast, and Cerophyll (less than 37 microns) should be added three times a week in a concentration equivalent to 4 or 5 mg/liter per week. One-fourth of the culture volume (along with the organisms) should be removed each week and fresh medium added to restore the original volume. These methods resulted in populations in which all size classes (age groups) were constantly evident.

C. bicuspidatus thomasi was best cultured using live Paramecium sp. cultures as food. About 1,000 of the protozoans/liter per week seemed to produce maximum standing crops at 15° C. A light:dark cycle of 12:12 hours was satisfactory. Cultures should be maintained in glass containers of at least 2-liter volume, in the synthetic medium of Sheer and Armitage, 1973. One-fourth of the culture medium (and organisms) was removed each week and fresh medium was added. Under these conditions, the number of organisms produced will be more variable and less predictable than those found in the C. sphaericus cultures. The proportion of each life stage in the total population may also fluctuate greatly. These factors plus the present inability to efficiently separate the various life stages make this species less desirable as live fish food or for bioassay test purposes.

In considering future work with zooplankton cultures, one of two directions seems inevitable. The first involves the use of small cultures containing

one to several individuals. The second would make use of mass culture techniques in which large containers would be used to produce thousands of organisms. There is no doubt that the small cultures, containing a single specimen, have yielded valuable information for a number of species. These small cultures, where individuals are subjected to direct observation, are particularly well suited for gaining certain kinds of data, (e.g., the number of eggs produced by a single female). However, after using these techniques over an extended period, this author questions the application of such results to entire populations inhabiting large and complex habitats. It seems more desirable, where possible, to use large cultures to test the effects of environmental variables (or pollutants) on populations rather than on individuals. This approach in culturing populations has worked particularly well with planktonic algae, and it seems equally well suited to zooplankton.

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APPENDIX

I. Recommended Procedures for Batch Culturing Chydorus sphaericus and Cyclops bicuspidatus thomasi.

1. Containers - Glass containers with a minimum volume of two liters and a high surface to volume ratio should be used. Larger containers with proportionate amounts of food could be maintained using the same methods and growing conditions.
2. Medium - Static cultures are reared in the synthetic medium described by Sheer and Armitage (1973):

Compound	Stock Solution	Add Per Liter
$MgSO_4$:7 H_2O	36.97 g/l	1.4 cc, liter
$NaHCO_3$	12.6 g/l	6 cc/liter
$KHCO_3$	6.4 g/l	1 cc/liter
$CaCl_2$:2 H_2O	2.5 g/l	8.8 cc/liter
K_2HPO_4	8.7 g/l	0.1 cc/liter
Na_2SiO_3:9 H_2O	18.1 g/l	1 cc/liter

3. Photoperiod - The photoperiod should be a standard 12 hour light-12 hour dark cycle. It may be desirable, when using the cultures to feed fish, to adjust the light-dark cycle to correspond to fish photoperiod. We found that two G.E., 20 watt, cool white fluorescent lamps produced satisfactory illumination.
4. Oxygen requirements - No aeration is necessary as long as the air-water interface is sufficiently large to provide maximum oxygen exchange. Usually this can be accomplished by using a container with a high surface to volume ratio. A periodic "spot check" of oxygen content should be taken.
5. Maintenance - At least one-fourth of the culture medium should be removed each week along with organisms and replaced with fresh synthetic medium. Our samples were removed with a vacuum device after thoroughly mixing the contents of the culture. Cultures were maintained in environmental chambers in which we were able to control photoperiods and temperatures.

6. Obtaining brood stock - A Wisconsin plankton net (with removable bucket) can be towed through the water to collect and concentrate the plankton. Taking care not to drain the bucket, it can be removed and the contents emptied into an insulated container containing water from the field source. This plankton can then be transported to the laboratory. After reconcentrating the plankton into a smaller container, desired species may be removed using a small pipette.

II. Methods for Chydorus sphaericus.

1. Brood stock - The brood stock can be obtained from field populations of C. sphaericus. Usually a dense population of this species can be found in shallow, temperate ponds during the warmer summer months. Cultures should initially be stocked with approximately 50 adults per liter.
2. Transfer and stocking cultures - Unlike some cladocerans, C. sphaericus can be easily transferred from one vessel to another with no adverse affects. This may be accomplished using a dissecting microscope and glass pipettes.
3. Temperature - Optimum temperatures for culturing C. sphaericus were not specifically determined, but cultures maintained at 15° C (± 1° C) were more productive than those at 10° or 20° C.
4. Food - Food consisting of equal proportions of LIV, brewer's yeast, and Cerophyll (less than 37 microns) should be added to the culture at least 3 times a week. The concentration added to the culture should be equivalent to 4-5 mg (dry weight) per liter per week. Specific sized foods can be obtained from stock supplies by first using a grinder to reduce the material to powder and then separating the powder into size categories with a micro-sifter. In our studies we used a micro-mill (Chemical Rubber Co.) to grind the foods to powder, and an Allen-Bradley "Sonic-Sifter" to separate the powder into various size categories.

   Stock solutions of the three foods were prepared by adding known quantities to distilled water. Each week fresh feeding solutions should be made from the stock solutions. Feeding solutions can be maintained for one week in the refrigerator without spoiling. However, stock solutions should be kept frozen prior to use.

III. Methods for Cyclops bicuspidatus thomasi.

1. Brood stock - C. bicuspidatus thomasi adults can usually be obtained from field populations from temperate lakes. Since this species is quite adaptable to a variety of environmental conditions, it may be found at varying periods during the year. We collected most of the brood stock for our experiments during the winter and spring.

   Cultures should initially be stocked with 50 mature C. bicuspidatus thomasi per liter (approximate male-female ratio 1:1). These plankton are relatively easy to handle and may be transferred from one container to another with a pipette.

2. Food - Cultures of Paramecium multimicronucleatum should be used as the food source for the C. bicuspidatus thomasi cultures. Each of our C. bicuspidatus thomasi was fed three times a week at a rate of 1,000 P. multimicronucleatum per liter per week.

   The protozoan cultures can be grown in 1 or 2 liters of synthetic medium containing Cerophyll (1 gram per liter) inoculated with Enterobacter aerogenes. These cultures should be maintained in subdued light at 25° C. To ensure that abundant supplies of protozoans are available, new cultures should be started periodically.

   Since densities of protozoan cultures (under these conditions) are quite variable, the quantity needed to feed the desired number of organisms into the cyclopoid cultures should be determined at each feeding. This may be done by counting a 1 ml aliquot of the protozoan culture and calculating the volume needed.

3. Temperature - Maximum standing crops during our experiments were produced at 15° C (± 1° C).